THE EFFECTS OF INHALED ASBESTOS ON PULMONARY MACROPHAGES: A MORPHOLOGICAL, FUNCTIONAL, AND BIOCHEMICAL STUDY

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Occupational exposure to asbestos clearly is associated with interstitial fibrotic lung disease. We are studying the initial cellular events following a brief asbestos exposure, in an animal inhalation model (Brody et al, Amer. Rev. Resp. Dis. 123:670, 1981). Following a one-hour exposure to chrysotile asbestos, translocation of fibers occurs through a variety of cell types, followed by development of lesions at alveolar duct bifurcations. In our inhalation model, we have studied the effects of chrysotile asbestos on populations of pulmonary macrophages exposed to the inhaled dust in situ. Subtle, but consistent changes occur in macrophages recovered by pulmonary lavage at several time periods following a 1 hour exposure. Morphological, functional and biochemical techniques have been utilized to document these early changes.

Fisher 344 rats were exposed, nose-only, to 16.5 mg/m^3 (respirable mass) of aerosolized chrysotile asbestos for one hour in an inhalation chamber. Equal numbers of animals were sham-exposed to air. Forty eight, 72 and 192 hours after exposure, pulmonary macrophages were recovered from animals by gentle lavage with Ca^{++} and Mg^{++}-free phosphate buffered saline. Cells were counted and viabilities determined using trypan-blue exclusion. One-half million cells per animal were then plated on Thermanox coverslips and placed in a CO_2 incubator for 45 minutes. These macrophage monolayers were either cultured for an additional 24 hours for biochemical studies, incubated with opsonized carbonyl iron particles for a phagocytosis assay, or prepared for quantitative ultrastructural studies.

No significant differences were found in cell numbers and viabilities of macrophages recovered from asbestos exposed and matched sham-exposed animals at the three time periods. The macrophage yields averaged 1.1 x 10^6 cells/6 lavages, while the viability of cells ranged from 91-97%.

Using light and electron microscopic techniques, we found that approximately 27% of the macrophages recovered after 48 hours contained asbestos particles, while only 10-15% of cells recovered after 8 days showed evidence of particle uptake.

We have established a morphologic model of macrophage injury. The results of preliminary experiments have suggested that macrophage toxicity correlates with a loss of surface ruffles and consequent smoothing of the cell membrane, following plating on plastic culture surfaces. In untreated cultured monolayers, ruffled macrophages (M^+) consistently comprised 70-80% of the population. We have increased the number of smooth-surfaced cells by incubating macrophages with crystalline silica, and in acidic culture conditions. Smooth macrophages (M^-) have a depressed phagocytic capacity in comparison to ruffled macrophages (M^+). Macrophages recovered from asbestos and sham exposed animals were plated on coverslips for 45 minutes and prepared for scanning and transmission electron microscopy. Companion monolayers were cultured with iron beads

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(.5-2 μm in diameter) for 1 hour. Secondary and backscatter imaging were utilized to quantitate iron uptake by individual cells. The results show that an increase in the percentage of smooth-surfaced macrophages from asbestos-exposed animals correlates with a decreased capacity of the lavaged cell population to phagocytize iron beads.

Biochemical studies were carried out on macrophages recovered from asbestos and sham-exposed rats. Cells were cultured for 24 hours and intra- and extracellular lysosomal enzymes were measured spectrophotometrically. We found increased intra- and extracellular levels of acid phosphatase, β-N-acetylglucosaminidase, and α-mannosidase in the cells from asbestos-exposed animals. These findings correlated with cytochemical experiments on asbestos and sham-exposed macrophages, where the chrysotile exposed cells displayed an increased degree of acid phosphatase staining.

A one-hour exposure to chrysotile asbestos causes subtle, but measurable changes in certain parameters of macrophage populations. Alterations in cell numbers and viability were not demonstrated. On the other hand, diminished phagocytic capacity correlated with increased numbers of smooth-surfaced cells, and increased enzyme activity was demonstrated in asbestos-exposed cells. Whether or not these changes play a role in the slowly progressing pathogenesis of asbestosis is a subject of further study.

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